

- 280 -

Claims:

1. A set of oligonucleotide probes, wherein said set comprises at least 10 oligonucleotides selected from:
5 an oligonucleotide as described in Table 1 or derived from a sequence described in Table 1, or an oligonucleotide with a complementary sequence, or a functionally equivalent oligonucleotide.
- 10 2. A set of oligonucleotide probes as claimed in claim 1 wherein said oligonucleotide probes are selected from: an oligonucleotide as described in Table 2 or derived from a sequence described in Table 2, or an oligonucleotide with a complementary sequence, or a functionally equivalent oligonucleotide.
15
- 20 3. A set of oligonucleotide probes as claimed in claim 1 wherein said oligonucleotide probes are selected from: an oligonucleotide as described in Table 4 or derived from a sequence described in Table 4, or an oligonucleotide with a complementary sequence, or a functionally equivalent oligonucleotide.
- 25 4. A set of oligonucleotide probes as claimed in any one of claims 1 to 3, wherein each probe in said set binds to a different transcript.
30
- 35 5. A set as claimed in any one of claims 1 to 4 consisting of from 10 to 500 oligonucleotide probes.
6. An oligonucleotide probe wherein said probe is selected from the oligonucleotides listed in Table 1, or derived from a sequence described in Table 1, or a complementary sequence thereof.
35
7. A set of oligonucleotide probes as claimed in any one of claims 1 to 5, or an oligonucleotide probe as

- 281 -

claimed in claim 6, wherein each of said oligonucleotide probes is from 15 to 200 bases in length.

8. A set of oligonucleotide probes as claimed in any one of claims 1 to 5 or 7 or an oligonucleotide probe as claimed in claim 6 or 7, wherein the transcript to which said probe binds is derived from a gene which is constitutively moderately or highly expressed.
9. A set of oligonucleotide probes as claimed in any one of claims 1 to 5, 7 or 8 or an oligonucleotide probe as claimed in any one of claims 6 to 8, wherein said probes are immobilized on one or more solid supports.
10. A set of oligonucleotide probes or an oligonucleotide probe as claimed in claim 9, wherein said solid support is a sheet, filter, membrane, plate or biochip.
11. A polypeptide encoded by the mRNA sequence to which an oligonucleotide as defined in claim 6 binds.
12. An antibody to a polypeptide as defined in claim 11.
13. A kit comprising a set of oligonucleotide probes immobilized on one or more solid supports as defined in claim 9 or 10.
14. A kit as claimed in claim 13 wherein said probes are immobilized on a single solid support and each unique probe is attached to different region of said solid support.
15. A kit as claimed in claim 13 or 14 further comprising standardizing materials.

- 282 -

16. The use of a set of probes as described in any one of claims 1 to 5 or 7 to 10 or a kit as described in any one of claims 13 to 15 to determine the gene expression pattern of a cell which pattern reflects the level of 5 gene expression of genes to which said oligonucleotide probes bind, comprising at least the steps of:

- a) isolating mRNA from said cell, which may optionally be reverse transcribed to cDNA;
- b) hybridizing the mRNA or cDNA of step (a) to a 10 set of oligonucleotides or a kit as defined in any one of claims 1 to 5, 7 to 10 or 13 to 15; and
- c) assessing the amount of mRNA or cDNA hybridizing to each of said probes to produce said pattern.

15 17. A method of preparing a standard gene transcript pattern characteristic of a disease or condition or stage thereof in an organism comprising at least the steps of:

- a) isolating mRNA from the cells of a sample of one 20 or more organisms having the disease or condition or stage thereof, which may optionally be reverse transcribed to cDNA;
- b) hybridizing the mRNA or cDNA of step (a) to a 25 set of oligonucleotides or a kit as defined in any one of claims 1 to 5, 7 to 10 or 13 to 15 specific for said disease or condition or stage thereof in an organism and sample thereof corresponding to the organism and sample thereof under investigation; and
- c) assessing the amount of mRNA or cDNA hybridizing 30 to each of said probes to produce a characteristic pattern reflecting the level of gene expression of genes to which said oligonucleotides bind, in the sample with the disease, condition or stage thereof.

35 18. A method of preparing a test gene transcript pattern comprising at least the steps of:

- a) isolating mRNA from the cells of a sample of

- 283 -

said test organism, which may optionally be reverse transcribed to cDNA;

5 b) hybridizing the mRNA or cDNA of step (a) to a set of oligonucleotides or a kit as defined in any one of claims 1 to 5, 7 to 10 or 13 to 15 specific for a disease or condition or stage thereof in an organism and sample thereof corresponding to the organism and sample thereof under investigation; and

10 c) assessing the amount of mRNA or cDNA hybridizing to each of said probes to produce said pattern reflecting the level of gene expression of genes to which said oligonucleotides bind, in said test sample.

19.- A method of diagnosing or identifying or monitoring 15 a disease or condition or stage thereof in an organism, comprising the steps of:

a) isolating mRNA from the cells of a sample of said organism, which may optionally be reverse transcribed to cDNA;

20 b) hybridizing the mRNA or cDNA of step (a) to a set of oligonucleotides or a kit as defined in any one of claims 1 to 5, 7 to 10 or 13 to 15 specific for said disease or condition thereof in an organism and sample thereof corresponding to the organism and sample thereof under investigation;

25 c) assessing the amount of mRNA or cDNA hybridizing to each of said probes to produce a characteristic pattern reflecting the level of gene expression of genes to which said oligonucleotides bind in said sample; and

30 d) comparing said pattern to a standard diagnostic pattern prepared as described in claim 17 using a sample from an organism corresponding to the organism and sample under investigation to determine the degree of correlation indicative of the presence of said

- 284 -

disease or condition or a stage thereof in the organism under investigation.

20. A method as claimed in any one of claims 17 to 19
5 wherein said mRNA or cDNA is amplified prior to step b).

21. A method as claimed in any one of claims 17 to 20
wherein the oligonucleotides and/or the mRNA or cDNA are labelled.

10 22. A method as claimed in any one of claims 17 to 21
wherein said probes are as defined in claim 3 and said disease is Alzheimer's disease.

15 23. A method as claimed in any one of claims 17 to 21
wherein said probes are as defined in claim 2 and said disease is breast cancer.

20 24. A method as defined in any one of claims 17 to 23,
wherein said set of oligonucleotides as defined in any one of claims 1 to 5, 7 to 10 or 13 to 15 are replaced with a set of oligonucleotides which are randomly selected, preferably from a cDNA library.

25 25. A method of preparing a standard gene transcript pattern characteristic of a disease or condition or stage thereof in an organism comprising at least the steps of:

30 a) releasing target polypeptides from a sample of one or more organisms having the disease or condition or stage thereof;

35 b) contacting said target polypeptides with one or more binding partners, wherein each binding partner is specific to a marker polypeptide (or a fragment thereof) encoded by the gene to which an oligonucleotide of Table 1 (or derived from a sequence described in Table 1) binds, to allow binding of said binding partners to said

target polypeptides, wherein said marker polypeptides are specific for said disease or condition thereof in an organism and sample thereof corresponding to the organism and sample thereof under investigation; and

5 c) assessing the target polypeptide binding to said binding partners to produce a characteristic pattern reflecting the level of gene expression of genes which express said marker polypeptides, in the sample with the disease, condition or stage thereof.

10

26. A method of preparing a test gene transcript pattern comprising at least the steps of:

a) releasing target polypeptides from a sample of said test organism;

15 b) contacting said target polypeptides with one or more binding partners, wherein each binding partner is specific to a marker polypeptide (or a fragment thereof) encoded by the gene to which an oligonucleotide of Table 1 (or derived from a sequence described in Table 1) binds, to allow binding of said binding partners to said target polypeptides, wherein said marker polypeptides are specific for said disease or condition thereof in an organism and sample thereof corresponding to the organism and sample thereof under investigation; and

20 c) assessing the target polypeptide binding to said binding partners to produce a characteristic pattern reflecting the level of gene expression of genes which express said marker polypeptides, in said test sample.

25 30 27. A method of diagnosing or identifying or monitoring a disease or condition or stage thereof in an organism comprising the steps of:

a) releasing target polypeptides from a sample of said organism;

35 b) contacting said target polypeptides with one or more binding partners, wherein each binding partner is specific to a marker polypeptide (or a fragment thereof)

- 286 -

encoded by the gene to which an oligonucleotide of Table 1 (or derived from a sequence described in Table 1) binds, to allow binding of said binding partners to said target polypeptides, wherein said marker polypeptides 5 are specific for said disease or condition thereof in an organism and sample thereof corresponding to the organism and sample thereof under investigation; and

10 c) assessing the target polypeptide binding to said binding partners to produce a characteristic pattern reflecting the level of gene expression of genes which express said marker polypeptides in said sample; and

15 d) comparing said pattern to a standard diagnostic pattern prepared as described in claim 25 using a sample from an organism corresponding to the organism and sample under investigation to determine the degree of correlation indicative of the presence of said disease or condition or a stage thereof in the organism under investigation.

20 28. A method as claimed in any one of claims 17 to 27 wherein said pattern is expressed as an array of numbers relating to the expression level associated with each probe.

25 29. A method as claimed in any one of claims 17 to 28 wherein said organism is a eukaryotic organism, preferably a mammal.

30 30. A method as claimed in claim 29 wherein said organism is a human.

35 31. A method as claimed in any one of claims 17 to 30 wherein the data making up said pattern is mathematically projected onto a classification model.

32. A method as claimed in any one of claims 17 to 31 wherein said disease is cancer or a degenerative brain

- 287 -

disorder.

33. A method as claimed in any one of claims 17 to 32 wherein said sample is tissue, body fluid or body waste.

5

34. A method as claimed in any one of claims 17 to 33 wherein said sample is peripheral blood.

10

35. A method as claimed in any one of claims 17 to 34 wherein the cells in the sample are not disease cells, have not been in contact with such cells and do not originate from the site of the disease or condition.

15

36. A method as claimed in any one of claims 19 to 35 for the diagnosis, identification or monitoring of two or more diseases, conditions or stages thereof in an organism, wherein said pattern produced in step c) is compared to at least two standard diagnostic patterns prepared as described in claim 17 or 25, wherein each standard diagnostic pattern is a pattern generated for a different disease or condition or stage thereof.

20

37. A method of identifying probes useful for diagnosing or identifying or monitoring a disease or condition or stage thereof in an organism, comprising the steps of:

25

- a) immobilizing a set of oligonucleotide probes, preferably as described hereinbefore, on a solid support;
- b) isolating mRNA from a sample of a normal organism (normal sample), which may optionally be reverse transcribed to cDNA;
- c) isolating mRNA from a sample from an organism, corresponding to the sample and organism of step (b), which is known to have said disease or condition or a stage thereof (diseased sample), which may optionally be reverse

30

35

- 288 -

- transcribed to cDNA;
- d) hybridizing the mRNA or cDNA of steps (b) and (c) to said set of immobilized oligonucleotide probes of step (a); and
- 5 e) assessing the amount of mRNA or cDNA hybridizing to each of said oligonucleotide probes to determine the level of gene expression of genes to which said oligonucleotide probes bind in said normal and diseased samples to generate a gene expression data set for each sample;
- 10 f) normalizing and standardizing said data set of step (e);
- 15 g) constructing a calibration model for classification, preferably using the statistical techniques Partial Least Squares Discriminant Analysis (PLS-DA) and Linear Discriminant Analysis (LDA);
- 20 h) performing JackKnife analysis and identifying those oligonucleotide probes which are required for classification of said disease and normal samples into their respective groups.